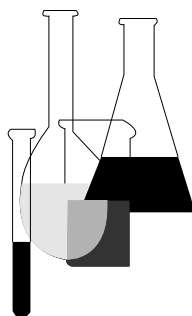




Fate, Transport and Transformation Test Guidelines

OPPTS 835.5045 Modified SCAS Test for Insoluble and Volatile Chemicals



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 835.5045 Modified SCAS test for insoluble and volatile chemicals.

(a) **Scope—(1) Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is 40 CFR 795.45 Inherent Biodegradability: Modified SCAS Test for chemical Substances That Are Water Soluble or Water Insoluble and Volatile..

(b) **Introductory information—(1) Prerequisites.** (i) Water solubility of the test chemical must be established.

(ii) The organic carbon content of the test chemical must be established.

(2) **Guidance information.** (i) Information on the relative proportions of the major components of the test chemical will be useful in interpreting the results obtained.

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) **Standard documents.** This Test Guideline has been based on the papers cited under paragraphs (e)(1) and (e)(2) of this guideline.

(c) **Method—(1) Introduction, purpose, scope, relevance, application and limits of test—(i) The method.** (A) The method is an adaptation of the Soap and Detergent Association Semi-Continuous Activated Sludge (SCAS) procedure for assessing the primary biodegradation of alkylbenzene sulphonate. The method involves exposure of the chemical to relatively high concentrations of microorganisms over a long time period (possibly several months). The viability of the microorganisms is maintained over this period by daily addition of a settled sewage feed.

(B) Since the conditions provided by the test are highly favorable to the selection and/or adaptation of microorganisms capable of degrading the test chemical, the procedure may also be used to produce microbial inocula adapted to selected chemicals for use in other tests. The test is applicable to organic chemicals that are water insoluble or water insoluble and volatile and that are not inhibitory to bacteria at the test concentration.

(ii) **Reference chemicals.** In some cases when investigating a new chemical, reference chemicals may be useful; however, specific reference chemicals cannot yet be recommended. Data on several chemicals used in interlaboratory tests are provided (see following Table 1.) primarily so

that calibration of the method may be performed from time to time and to permit comparison of results when another method is employed.

Table 1.—Examples of Results of SCAS Test on Various Chemicals Used in the OECD/EEC Interlaboratory Test

Test chemical	O _T (mg/L)	O _t – O _c (mg/L)	Percent biodegradation/ bioelimination
4-Acetylamino benzene sulfonate.	17.2	2.0	85
Tetrapropylene benzene sulfonate.	17.3	8.4	51.4
4-Nitrophenol	16.9	0.8	95.3
Diethylene glycol	16.5	0.2	98.8
Aniline	16.9	1.7	95.9
Cyclopentane tetracarboxylate ..	17.9	3.2	81.1

Duration of test is 40 days, except 120 days for cyclopentane tetracarboxylate.

(iii) **Principle of the test method.** (A) Activated sludge from a sewage treatment plant is placed in an aeration (SCAS) unit. The test chemical and settled domestic sewage are added, and the mixture is aerated for 23 hours. The aeration is then stopped, the sludge is allowed to settle, and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a further aliquot of test chemical and sewage and the cycle is repeated.

(B) This method requires use of a chemical-specific analytical technique or ¹⁴C-labeled test chemical. The purpose of the method is to determine the fate of the test chemical in a conventional activated sludge treatment plant. To this end, a complete mass balance for the test chemical is established by quantifying parent chemical in settled effluent sludge solids (insoluble test chemicals whether volatile or not), effluent plus solids (insoluble test chemicals whether volatile or not), and off gases (volatile test chemicals only). The identification and quantification of degradation products in all phases are recommended, but not required.

(iv) **Quality criteria—(A) Reproducibility.** When primary biodegradation is considered, very precise data are obtained for chemicals that are extensively degraded. The results reported in the reference under paragraph (e)(1) of this guideline suggest 95-percent confidence limits of less than ±3 percent, and this includes interlaboratory tests. As would be expected, wider confidence limits are obtained for less biodegradable chemicals.

(B) **Possibility of standardization.** Since the method uses a feed of settled sewage, absolute standardization is not possible unless this feed were replaced by synthetic sewage. However, since the method is designed

to give an indication of the biodegradability potential of a chemical and is not a simulation test such standardization is unnecessary.

(C) **Possibility of automation.** Automation of this method would be possible but would be expensive. As the method is not labor intensive, the exercise would offer few advantages.

(2) **Description of the test procedure—(i) Preparations.** (A) The aeration units are cleaned and fixed in a suitable support. The air inlet tubes are connected to the supply manifold. A small laboratory-scale air compressor is used to aerate the units, and the air is presaturated with water to reduce evaporation losses from the units.

(B) If the test chemical is volatile, exhaust gases from the aeration units should be passed through a suitable trap (such as Amberlite XAD-4, Rohm and Haas, Philadelphia, PA) to remove volatilized organics.

(C) A sample of mixed liquor from an activated sludge plant treating predominantly domestic sewage is obtained. Approximately 150 mL of the mixed liquor are required for each aeration unit.

(D) The organic carbon analyzer is calibrated using potassium hydrogen phthalate.

(E) Stock solutions of the test chemicals are prepared: The concentration normally required is 400 mg/L as organic carbon which gives a test chemical concentration of 20 mg/L carbon at the start of each aeration cycle if no biodegradation is occurring.

(F) If the test chemical is insoluble in water at 400 mg/L it may be necessary to use ultrasound dispersion to obtain a uniform stable suspension. Alternatively, test chemical may be added directly to the aeration units.

(G) The organic carbon content of the stock solutions is measured.

(ii) **Test conditions.** A high concentration of aerobic microorganisms is used, and the effective detention period is 36 hours. The carbonaceous material in the sewage feed is oxidized extensively within 8 hours of the start of each aeration cycle. Thereafter, the sludge respire endogenously for the remainder of the aeration period, during which time the only available substrate is the test chemical unless this is also readily metabolized. These features, combined with daily reinoculation of the test when domestic sewage is used as the medium, provide highly favorable conditions for both adaptation and biodegradation.

(iii) **Performance of the test.** (A) A sample of mixed liquor from a suitable activated sludge plant is obtained and aerated during transportation to the laboratory. Each aeration unit is filled with 150 mL of mixed

liquor, and aeration is started. After 23 h, aeration is stopped, and the sludge is allowed to settle for 45 min. The tap is opened, and 100 mL of the supernatant liquor is withdrawn. A sample of settled domestic sewage is obtained immediately before use, and 100 mL is added to the sludge remaining in each aeration unit. Aeration is started anew. At this stage no test chemicals are added, and the units are fed daily with domestic sewage only until a clear supernatant liquor is obtained on settling. This usually takes up to 2 weeks, by which time the dissolved organic carbon in the supernatant liquor at the end of each aeration cycle should be less than 12 mg/L.

(B) At the end of this period the individual settled sludges are mixed, and 50 mL of the resulting composite sludge is added to each unit.

(C) One hundred milliliters of settled sewage are added to the control units, and 95 mL of settled sewage plus 5 mL of the appropriate test chemical stock solution or suspension (400 mg organic carbon/L) to the test units. If test chemical is added directly to aeration units, 100 mL of settled sewage is added, as in the control units.

(D) Aeration is started again and continued for 23 h. The sludge is then allowed to settle for 45 min and the supernatant drained off and analyzed for parent chemical. Before analysis the liquors are filtered through washed 0.45 μm membrane filters and centrifuged. Temperature of the sample must not exceed 40 °C while it is in the centrifuge.

(E) If the test chemical is insoluble or expected to sorb significantly to sludge solids, settled sludge is also collected by an appropriate means (such as centrifugation) and extracted to remove test chemical, and the extract is analyzed for parent chemical.

(F) If the test chemical is volatile, traps for removing volatile organics from exhaust gases are also extracted and the extracts analyzed for parent chemical.

(G) The fill and draw procedure under paragraphs (c)(2)(iii)(C) through (c)(2)(iii)(F) of this guideline is repeated daily throughout the test.

(H) Before settling, it may be necessary to clean the walls of the units to prevent the accumulation of solids above the level of the liquid. A separate scraper or brush is used for each unit to prevent cross contamination.

(I) The length of the test for chemicals showing little or no biodegradation is indeterminate, but experience suggests that this should be at least 12 weeks.

(d) Data and reporting—(1) Treatment of the results. (i) The concentration of parent chemical in settled effluent sludge solids (insoluble test chemicals whether volatile or not), effluent plus solids (insoluble test

chemicals whether volatile or not), and off-gases (volatile test chemicals only) is plotted versus time for the test units. As biodegradation is achieved the level of the test chemical will decrease and approach a steady state. Once the levels of the test chemical are found to be constant over three consecutive measurements, three further measurements are made.

(ii) An example of the application of specific analytical technique to the SCAS test is discussed in the reference in paragraph (e)(2) of this guideline.

(e) **References.** The following references should be consulted for additional background information on this test guideline.

(1) A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulfonate and Linear Alkylate Sulfonate. *Journal of the American Oil Chemists Society* 42:986 (1965).

(2) Games, L.M. et al. Fate and distribution of a quaternary ammonium surfactant octadecyltrimethylammonium chloride (OTAC), in wastewater treatment. *Environmental Science and Technology* 16:483–488 (1982).